# ELECTROPHYSIOLOGY OF THE OLFACTORY NERVE OF THE PIKE, *ESOX LUCIUS*: A PILOT STUDY ON OPTIMAL EXPERIMENTAL CONDITIONS

by

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**ABSTRACT**. - Electrophysiological recordings in the olfactory nerve of the European pike *Esox lucius* were done as early as at the beginning of the 20th century, but since then, the nerve has not been extensively studied. It contains 4 to 6 millions unmyelinated sensory axons, with a relatively homogenous population of sizes, more than 95% have diameters of about 0.2 $\mu$m. The aim of our work concerned the keeping conditions under which reproducible electrophysiological responses can be obtained, and some of the electrophysiological properties of these unmyelinated sensory axons. Particular attention has been given to the dissection of the pike olfactory nerve. We have adapted the technique described by von Muralt *et al.* (1976) to obtain the nerves more quickly and to prolong their survival. For the latter aim, we have found that a medium, used to keep excised *Xenopus* oocytes for many days, kept 69% of the nerves functional for up to 3 days. The diphasic compound action potential (CAP) of the pike olfactory nerve propagated at a velocity of $12 \pm 0.5$ cm/s ($n = 37$) and was suppressed by tetrodotoxin (10 $\mu$M), known to block voltage-activated sodium channels. Nerve CAP kinetics were noticeably modified when tested after about 3 h in the medium at room temperature. However, the nerves produced consistent CAP during 30-60 min in the recording chamber in air. While preliminary, our results indicate that the olfactory nerve of the European pike, *Esox lucius*, by its survivability may be a suitable model for electrophysiological and pharmacological studies of unmyelinated sensory axons.

**RÉSUMÉ**. - Électrophysiologie du nerf olfactif du brochet, *Esox lucius*: une étude pilote sur les conditions expérimentales optimales.

Bien que les premiers enregistrements électrophysiologiques du nerf olfactif du brochet européen *Esox lucius* aient été effectués dès le début du 20ème siècle, ce nerf n'a cependant pas été beaucoup étudié. Il est constitué de 4 à 6 millions d'axones sensoriels amyéliniques dont la taille est relativement homogène puisque plus de 95% de ces axones ont un diamètre d'environ 0,2 $\mu$m. Le but de notre travail concernait les conditions de survie des nerfs permettant d'obtenir des réponses électrophysiologiques reproductibles ainsi que l'étude de certaines des propriétés électrophysiologiques de ces axones sensoriels amyéliniques. Une attention particulière a été apportée à la dissection du nerf olfactif de brochet. Nous avons adapté la technique décrite par von Muralt *et al.* (1976) de façon à obtenir les nerfs plus rapidement et à prolonger leur survie. Nous avons ainsi trouvé qu'un milieu, habituellement employé pour conserver pendant plusieurs jours les ovocytes prélevés sur des Xénopes, permettait de garder vivants, 3 jours durant, 69% des nerfs disséqués. Le potentiel d'action global biphasique (PAG) du nerf olfactif de brochet se propageait à une vitesse de $12 \pm 0,5$ cm/s ($n = 37$) et était supprimé par la tétrodotoxine (10 $\mu$M) connue pour bloquer les canaux sodium activés par le potentiel de membrane. Le décours du PAG était notablement modifié lorsque les nerfs étaient testés après avoir été maintenus

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dans le milieu à température ambiante pendant plus de 3 h. Cependant, les nerfs produisaient des PAG reproductibles lorsqu'ils étaient à l'air dans la cuve d'enregistrement pendant 30 à 60 min. Bien que préliminaires, nos résultats indiquent que le nerf olfactif du brochet européen, *Esox lucius*, par sa capacité de survie, peut être un excellent modèle pour les études électrophysiologiques et pharmacologiques des axones sensoriels amyéliniques.

Key words. - Esocidae - *Esox lucius* - Olfactory nerve - Unmyelinated sensory axons - Electrophysiology.

The pike olfactory nerve was used for some of the earliest electrophysiological studies which were to lead to our present understanding of excitable tissues (see Sowton, 1900; Garten, 1903). This nerve again attracted the interest of neurobiologists in the 1960's due to its 4 to 6 millions unmyelinated sensory axons, more than 95% of them having diameters of about 0.2 $\mu$m and the rest averaging 0.6 $\mu$m (Gasser, 1956; von Muralt *et al.*, 1976; Kreutzberg and Gross, 1977). In addition to its large number of small diameter axons, the pike olfactory nerve, similarly to the garfish olfactory nerve, has a much higher proportion of axonal membrane per unit nerve weight than, for example, the rabbit *vagus* nerve (Gasser, 1956; Keynes and Ritchie, 1965; Easton, 1971; von Muralt *et al.*, 1976; Kreutzberg and Gross, 1977).

The large proportion of active axonal membrane provided the basis for many investigations using optical and thermal measurement techniques to study the physical basis for the propagated action potential (Howarth *et al.*, 1975; von Muralt *et al.*, 1976; Georgescauld and Duclohier, 1978; Savoie *et al.*, 1986). Perhaps because of its delicate nature, the electrophysiological and pharmacological properties of the pike olfactory nerve are not yet well known.

The experiments to be described here were done to examine the difficulties that may be encountered in dissecting the pike olfactory nerve and the conditions under which its electrophysiological responsiveness can be maintained.

## MATERIALS AND METHODS

European pike, *Esox lucius*, weighing 1 to 1.5 kg were either purchased from the "Pisciculture Vasseur" (Les Cailleaux, Beaumont-les-Autels, France) or were kindly donated by the "Ecole Nationale des Gardes-pêche du Conseil Supérieur de la Pêche" (Boves, France). The animals were kept in tanks filled with aerated tap water in our laboratory animal facilities without feeding for no more than 8-10 days before they were sacrificed. An important detail is to provide an adequate cover on the tanks as these fish will attempt to escape the confinement by jumping out.

### Dissection

Olfactory nerves were dissected out according both to the description of the preparation given by Sowton (1900) and, with some modifications, to the method reported by von Muralt *et al.* (1976). A pike was decapitated behind the gills and the lower jaw was removed with a large knife. The head was then cooled in ice for about 30 min to reduce metabolic processes during the dissection. The head was fixed flat on a polystyrene board with large pins passing through the gill flaps, in back, and the upper bone of the mouth,

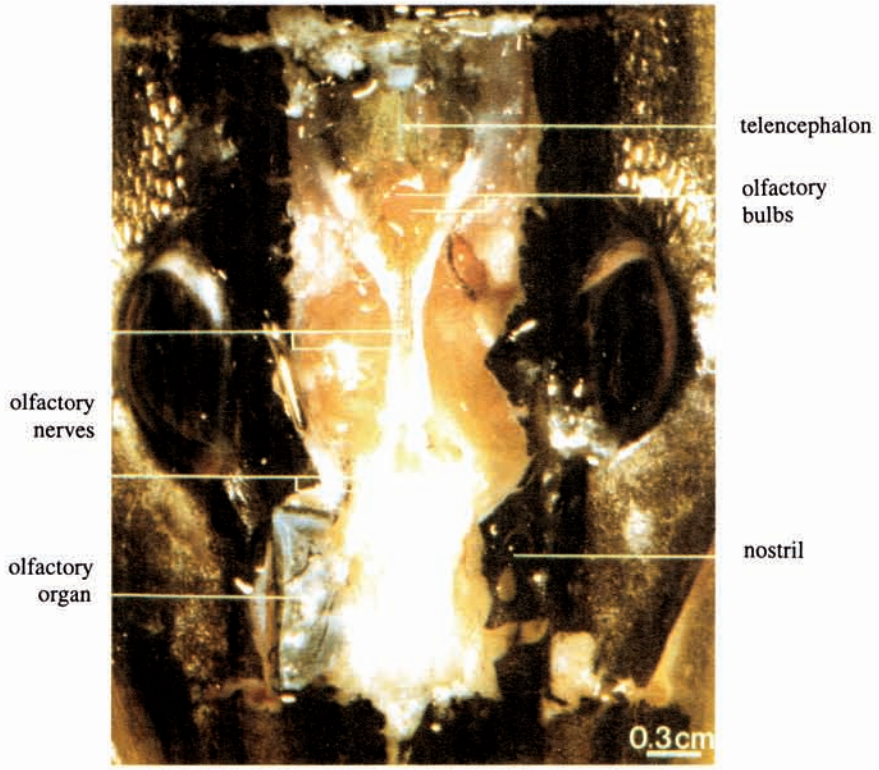


Fig. 1. - The olfactory system of the pike, *Esox lucius*. The rectangular opening in the head exposes both olfactory nerves as well as their end organs as labeled. The anterior part of the nerves disappears into a soft tissue just before the sensory organ in the nostril.

in front. With a cutting disk in a dentist's drill or a hacksaw blade, the skin and skull were cut first, transversely at the level of a "V" formed by the skull and visible on the surface of the skin. At this position, the skull was cut near the junction of the optic tecta and the fore-brain. A second transversal cut was then made at a level just anterior to the opercula of the nasal openings. Two longitudinal cuts, just medial from the eyes, connected the two transversal cuts to form an almost rectangular series of sectioned skull. After having separated the bony skull from the underlying cartilaginous matrix using a screw driver point, the olfactory nerves were visible through the cartilage.

The two olfactory nerves run through tunnels in the cartilage along with the trigeminal nerve and blood vessels, but usually were prominently visible (see Fig. 1). The overlying cartilage was then removed by slicing, parallel to the nerve, with a scalpel. It is advisable to make only thin slices to start but with experience, thicker slices may be made. This work was done using a dissecting microscope with illumination from the optical axis or laterally placed light sources.

The completely exposed nerves from the sensory end organ to the olfactory bulb appear as shown in figure 1. The lower parts of the nerves are encased in a gelatinous mass (white parts in Fig. 1) which can be removed. The sensory organ was separated from the nostril by cutting through its junction with the skin. The nerve could then be lifted

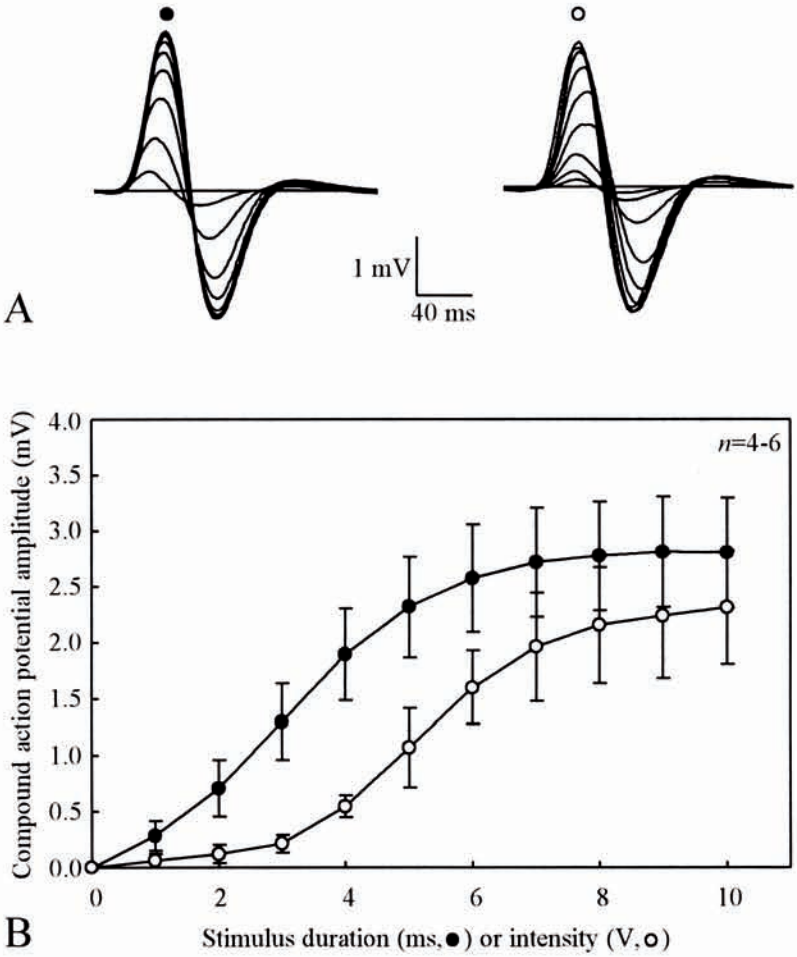


Fig. 2. - Effects of stimulation parameters on the compound action potential (CAP). **A**: Typical CAP recorded from the pike olfactory nerve stimulated with either pulse amplitudes of ,0-10 V and 7 ms duration (left traces, •), or pulse duration's of 0-10 ms and 7 V amplitude (right traces, o). **B**: Peak amplitudes of the first phase of the CAP as a function of either the duration or the voltage of the stimulus on the same abscissa. Means ± S.E.M. of data from 4-6 nerves.

out of its channel up to the olfactory bulb, by holding the bit of remaining nostril skin with tweezers. Care should be exercised to avoid stretching the nerve and any sign of resistance to lifting should be eliminated by further dissecting the cartilage. The dissected nerves were usually about 1 mm in diameter and 2-3 cm in length and could be obtained in about 30 min.

**Medium**

After dissection, the olfactory nerve together with the bulb and sensory organ was kept in one of the two bathing media at 4°C between recording sessions and overnight.

The medium was either the "pike" solution containing 120 mM NaCl, 4 mM KCl, 11 mM $CaCl_2$, 10 mM HEPES and 5 mM glucose, pH 6.8 adjusted with HCl (von Muralt *et al.*, 1976) or the "*Xenopus*" medium originally formulated for keeping excised *Xenopus* oocytes and containing 82.5 mM NaCl, 2.5 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 1 mM $Na_2HPO_4$ and 5 mM HEPES, pH 7.3 adjusted with NaOH (Wallace *et al.*, 1973).

### Electrophysiology

Electrophysiological stimulation and recording were done at room temperature (20-22°C) using conventional electrophysiological techniques. Briefly, the olfactory nerve segment was gently placed on two pairs of uninsulated platinum wires (0.5 mm diameter) fixed in a Plexiglas chamber and spaced 9.1 mm apart. One pair, spaced 2.3 mm apart, was connected to a stimulator which delivered rectangular pulses variable in amplitude and duration. The nerve was oriented so that stimulation occurred at the part nearest the sensory organ. The other pair, spaced 8.7 mm apart, was connected to the inputs of a high gain differential input amplifier for recording the compound action potential (CAP). The output of the amplifier was then digitized and stored using a computer with Axon Pclamp 5.5 hardware and software. A fifth platinum wire, fixed midway between the stimulating and recording pairs of wires, was connected to the ground. It is worth noting that the suspended nerve was neither bathed nor superfused during the recording period to avoid the shunting effects of the medium. However, the Plexiglas chamber was covered and the humidity inside was ensured by wads of cotton soaked with either "pike" or "*Xenopus*" solution.

Whenever possible, data are given as the mean ± S.E.M. of n olfactory nerves.

## RESULTS

A pike olfactory nerve produced a diphasic compound action potential in response to a single electrical stimulus (see Fig. 2A). Each phase was about 40 ms in duration and showed a single form, probably reflecting the relatively homogeneous size distribution of unmyelinated axon diameter known to constitute this type of nerve (see Introduction). The two phases of the CAP were consistently suppressed in nerves pre-treated for 8-10 min in "pike" or "*Xenopus*" solution containing 10 $\mu$M tetrodotoxin (data not shown), known to block voltage-activated sodium channels. This indicates that voltage-activated sodium channels are involved in the propagation of the CAP of the pike olfactory nerve, as in other excitable membranes.

The amplitude of the CAP was dependent on the intensity of the stimulus. With increasing voltages and fixed duration, the CAP increased to a plateau value and remained constant for further voltage increases (Fig. 2). With increasing duration of a fixed voltage stimulus pulse, the CAP amplitude behaved similarly (Fig. 2). The fact that the CAP amplitude attained a plateau indicated that all the fibers which could be stimulated, under our experimental conditions, were recruited by stimulus pulses of about 8-9 V and 7-8 ms. Under these conditions, the maximum amplitude of the CAP was found to be $2.77 \pm 0.15$ mV ($n = 37$) and to propagate at a velocity of $12 \pm 0.5$ cm/s ($n = 37$). These data were obtained from either freshly dissected nerves or from those dissected no more than 24 h previously and kept in the "pike" or "*Xenopus*" solution at 4°C. In two nerves, the CAP amplitudes showed a maximum at about the same stimulus parameters but then decreased with increasing stimulating voltages (data not shown).

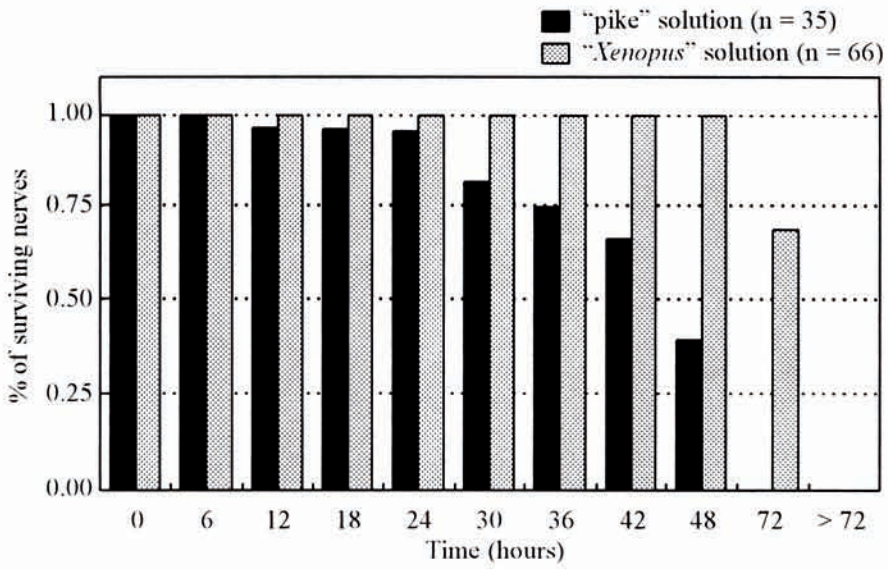


Fig. 3. - Nerve survival. Dissected nerves were tested for responsiveness at different times after being kept at 4°C between recording sessions. Those having a CAP of at least 2 mV, in response to a stimulus (10 V, 7 ms), were taken as surviving. The number of surviving nerves is expressed as a percentage of the total number of nerves tested in a recording session and plotted against their time after dissection. The data were from nerves kept in either the "pike" solution (n = 35, black bars) or the "*Xenopus*" solution (n = 66, grey bars).

Despite the fact that the pike olfactory nerves were neither bathed nor superfused during recording (see Materials and methods), the kinetics of their CAP were not noticeably modified during the 30-60 min (n = 10) in the recording chamber at room temperature. Similar results were obtained when nerves were kept at room temperature in the "pike" or "*Xenopus*" solution for no more than about 3 h between recording sessions of 10-15 min duration (n = 14). In contrast, nerve CAP were markedly reduced in amplitude and increased in duration when tested after 3 h in the medium at room temperature (n = 14). Nerves survived for more than this time only if they were kept at 4°C (see below).

In addition to the temperature, nerve survival was favored by storing them in the "*Xenopus*" solution rather than in the "pike" solution. It was found that 100% of the nerves kept in the "*Xenopus*" solution survived for about 48 h and 69% for about 72 h (n = 66). When kept in the "pike" solution for 48 h, only 40% of the nerves (n = 35) could produce a CAP and at 72 h none were viable. None of the nerves maintained in either solution for more than 72 h were functional. These data are summarized in the figure 3.

## DISCUSSION

The above results show that the olfactory nerves of the European pike *Esox lucius* were obtained with relative ease and showed a good survivability. The finding that survival times at 4°C were longer in "*Xenopus*" medium may have been due to the presence of $Na_2HPO_4$ in this medium, which may have contributed to the respiratory pH regulation. This may be a more important factor for survival than the presence of glucose which was in the "pike" medium. However, other components may also contribute to maintain a good survivability of nerves. In fact, it is likely that the ideal medium needs to be more complex than the two tested media since the survival time at room temperatures was about 3 h in either medium. Further experiments are needed to establish the composition of such an ideal medium.

The exposed sensory epithelia, nerves and olfactory bulbs *in situ* (see Fig. 1), appear to be an ideal preparation for studying an intact olfactory system almost *in vivo*. With more advanced electrophysiological techniques, it may be possible to obtain detailed data concerning the properties of the individual nerve fibers as well as those of the synapses in the olfactory bulb. The very high excitable membrane content of this nerve also makes it very suitable for studies on the structure of nerve membranes using such techniques as X-ray diffraction. Therefore, it would be worthwhile to determine whether the entire system is functional and for how long in the dissected conditions. However, as already found by Gasser (1956), the morphology of the sensory end organ and its exiting fibers will not lend themselves easily to detailed study.

In conclusion, our results indicate that the olfactory nerve of the European pike, *Esox lucius*, by its survivability, represents a suitable model to study the electrophysiological and pharmacological properties of sensory unmyelinated axons.

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